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The Subunit Structure of Elongation Factor 1 from *Artemia*

WHY TWO α-CHAINS IN THIS COMPLEX?*

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Elongation factor 1 (EF-1) regulates the specific interaction of aminoacyl-tRNA with the ribosome during the elongation phase of protein biosynthesis. Although individual functions of its separate chains have been well defined, to date there is hardly information about the structure and function of the whole complex. We describe here the complete subunit structure of elongation factor 1, and discuss its change during development of *Artemia*. Elongation factor 1 consists of a pentameric complex, composed of four different subunits α, β, γ, and δ in a molar ratio of 2:1:1:1. Although one molecule of EF-1α dissociates easily from the complex EF-1$\alpha_2\beta\gamma\delta$ under the influence of aminoacyl-tRNA and GTP, the second molecule of EF-1α was found to remain firmly attached. Thus, in eukaryotic protein synthesis, movement of transfer RNAs to the ribosome seems under the influence of two distinct molecules of EF-1α, a result possibly related to the presumed consumption of two molecules of GTP by EF-Tu during the elongation step of prokaryotic protein synthesis.

During the last decade, regulation of protein synthesis has been studied mostly at the level of initiation. Part of this is due to the success of the hemoglobin model (1) and the intuitive feeling that control of translation occurs as a single event at the start of the messenger RNA rather than during its whole readout. More recently it has become increasingly clear that the role of the elongation step in this process has been underestimated. In particular, the concept that EF-1[1] is involved in the maintenance and regulation of protein synthesis gets more and more accepted (2–7) and is a recurrent theme in a variety of processes, as diverse as embryogenesis (8, 9), cell division (10), cell proliferation (11), aging (12, 13), oncogenic transformation (14, 15), and the cellular organization of the cytoskeleton (16, 17). On the other hand little is known specifically about the molecular details of how, for example, EF-1 checks the correct reactivity of incoming tRNAs on the ribosome with the exception of what is known for bacterial EF-Tu (18). The possibility also remains that EF-1α serves in other processes than translation, for instance in transcription (19).

EF-1 consists of two functionally distinct parts. EF-1α catalyzes the binding of aminoacyl-tRNA to the ribosome under hydrolysis of GTP. The heteromeric complexes EF-1βγ and EF-1βγδ function in recycling the inactive EF-1α·GDP intermediate to the active GTP-bound form by stimulating guanine nucleotide exchange on EF-1α. Both subunits, EF-1β and EF-1δ, display the same degree of exchange activity (20), but differ with respect to the mode and strength of their interaction with EF-1γ.

Our laboratory has been interested for a long time in the role of EF-1 during development of *Artemia* (21). In the cell, EF-1 often occurs as several high molecular weight entities, referred to as EF-1H. During development of *Artemia* or wheat embryos, the dissociated form of EF-1H, EF-1α, becomes dominant in cell extracts (8, 22). The latter finding has been interpreted as evidence for EF-1H being a storage form during periods of dormancy or cryptobiosis (8, 23). However, in analogy with prokaryotic EF-Tu·EF-Ts (24), EF-1H has to dissociate into free EF-1α and the nucleotide exchange part in order to react with aminoacyl-tRNA and the ribosome (25). This transient dissociation, however, should not be taken as proof that during protein synthesis the guanine nucleotide exchange process necessarily takes place completely outside the region of the ribosome (26, 27). In fact it has been suggested that in higher eukaryotes transfer RNAs, aminoacyl-tRNA synthetases and elongation factors form a kind of "super structure" surrounding the ribosome as a cloud, thereby promoting the efficiency of protein synthesis of the eukaryotic cell (28–30). In order to understand better the kinetics and interplay of the different steps of elongation and its repercussions on the activation of protein synthesis especially during cyst-to-nauplius transition of *Artemia*, we started a systematic study on the quaternary structure of EF-1 (20, 31, 32) and the effects of transfer RNA and ribosomes thereupon. We delineated the mode of interaction between the different subunits of EF-1 by using limited protease cleavage, electrophoresis under nondenaturing conditions, microsequencing, and Western blotting.

As a result, we present for the first time a model for the quaternary structure of EF-1, including the interaction sites between the different subunits. A major finding in this paper is the presence of two molecules of EF-1α in EF-1 and each of the two EF-1α molecules behaving differently with respect to its substrates, aminoacyl-tRNA and GTP.

MATERIALS AND METHODS

Purification of Elongation Factors—EF-1 complexes were purified from *Artemia* cysts (San Francisco Bay Brand Inc., CA). Homogenization, aqueous two-phase separation, and hydroxyapatite chromatography were performed as described earlier (23). Fractions were analyzed by SDS-PAGE, and those containing EF-1 were pooled, diluted with 2.5 volumes of 10% (v/v) glycerol, 10 mM 2-mercaptoethanol, and concentrated on a small hydroxyapatite (BIO-GEL HTP, Bio-Rad) column (1 × 10 cm), previously equilibrated with 50 mM potassium phosphate, pH 7.4, 10 mM 2-mercaptoethanol, and 10% (v/v) glycerol. Bound protein was eluted with the same buffer, but containing 0.35 M potassium phos-

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[1] The abbreviations used are: EF, elongation factor; tRNA, transfer RNA; SH2, Src homology domain 2; FPLC, fast protein liquid chromatography; PAGE, polyacrylamide gel electrophoresis; GMP-PCP, guanylyl β,γ-methylenediphosphonate.

phate. A final concentration of about 50 mg/ml protein was achieved by vacuum dialysis against 25 mM Tris-HCl, pH 7.5, 50 mM KCl, 10 mM 2-mercaptoethanol, 1 mM magnesium acetate, and 10% (v/v) ethylene glycol. 100-μl aliquots were then applied to 4-ml 10–25% linear sucrose gradients in 20 mM Tris-HCl, pH 7.5, 100 mM KCl, 10 mM 2-mercaptoethanol, and 1 mM magnesium acetate, and centrifuged for 17 h at 43,000 rpm at 0 °C in a Beckman SW60 rotor. Tubes were pierced at the bottom, fractions of 6 drops (0.30 ml) were collected, and aliquots analyzed by SDS-PAGE and PAGE under nondenaturing conditions. Both complexes were free of bound guanine nucleotide, as determined by high pressure liquid chromatography (25).

Pure subunits, EF-1α, EF-1β, and EF-1γ, and the exchange complexes EF-1βγ and EF-1βγδ were isolated from *Artemia* cysts as described previously (16, 33).

Pure EF-1δ was recovered from the phenyl-Sepharose fractions eluting just after the EF-1βγ complex (16). Fractions were pooled on the basis of purity, especially avoiding contamination with EF-1βγ, and concentrated to about 0.1 mg/ml protein by vacuum dialysis against 5 mM sodium pyrophosphate, pH 6.7, 2.5 mM $MgCl_2$, and 0.3 M NaCl.

EF-1αδ complex was reconstituted from pure EF-1α and EF-1δ and concentrated on a hydroxyapatite column as described above.

EF-1 Activity Assays and Protein Concentration—Guanine nucleotide exchange activity, tRNA binding to ribosomes, and poly(phenylalanine) synthesis were determined precisely as described previously (33). Protein concentration was determined according to Lowry *et al.* (34).

Reconstitution—Reconstitution of EF-1 complexes was achieved by mixing, in a total volume of 20 μl, the different subunits and exchange complexes as indicated in figures. After an incubation of 15 min at 0 °C, the solution was mixed with 10 μl of electrophoresis sample buffer and subjected to PAGE under nondenaturing conditions using 4.5% polyacrylamide gels as described previously (31). Gels were stained with Coomassie Blue. Occasionally, the composition of the different protein bands was analyzed by SDS-PAGE (35) as follows. Protein bands were excised from nondenaturing gels, equilibrated with SDS-PAGE sample buffer (30 min), heated for 5 min at 95 °C, and applied to an SDS-polyacrylamide gel.

Determination of Subunit Stoichiometry—The ratio of α, β, γ, and δ in native EF-1 complexes was determined by the following procedure. The different complexes of EF-1 were separated into their constituent subunits by SDS-PAGE. After Coomassie Blue staining, gels were scanned with a Joyce-Loebl microdensitometer and the values obtained for each of the different subunits of EF-1 compared with those obtained from the same gel for single subunits, their protein concentration determined via the more absolute method of amino acid analysis (36). Calibration curves were linear for all four subunits. Molar ratios were estimated using molecular mass values of 49, 26, 46, and 28 kDa for α, β, γ, and δ, respectively. Reconstituted complexes were first recovered from nondenaturing gels and then analyzed as above.

The ratio of EF-1 subunits in crude *Artemia* extracts was estimated from Western blots (see next section), which were stained using the enhanced chemiluminescence technique (ECL, Amersham). The density readings of the EF-1α, EF-1β, EF-1γ, and EF-1δ bands were compared to those obtained by serial dilution of purified EF-$1\alpha_2\beta\gamma\delta$, its ratio taken as 2:1:1:1 (Table II).

Determination of the Binding Site for EF-1δ on EF-1βγ—5-μg amounts of EF-1βγ were digested with 0.25 μg of trypsin for 1 h at 0 °C in a 10-μl reaction mixture containing 20 mM Tris-HCl, pH 7.5, 0.1 mM EDTA, 10 mM 2-mercaptoethanol, and 25% (v/v) ethylene glycol (31). Further proteolysis was inhibited by the addition of 0.5 μg of Trasylol. The digested protein mixture was then added to EF-1δ as indicated, and reconstitution followed by analysis on a 7.5% nondenaturing gel. Proteins were either stained with Coomassie Blue or immunostained after semi-dry blotting for 2 h at 150 mA onto nitrocellulose using 50 mM Tris base, 50 mM glycine, 0.1% SDS, and 20% (v/v) methanol (37). Blocking and antibody incubations were performed at 37 °C in a buffer composed of 50 mM Tris-HCl, pH 7.4, 550 mM NaCl, 1% (w/v) Nonidet P-40, and 10 mg/ml bovine serum albumin. Washing of membranes was carried out in the same buffer but without albumin. Under these conditions all primary antibodies recognized specifically the cognate bands on Western blots of EF-$1\alpha_2\beta\gamma\delta$. Polyclonal anti-EF-1β and anti-EF-1δ antibodies were raised in rabbit, whereas monoclonal antibodies toward EF-1γ were from mice. Secondary antibodies were conjugated with horseradish peroxidase (Amersham). Positive reactions were visualized using the H_2O_2-diaminobenzidine reaction.

Gel Filtration and Dissociation of EF-1—80-μg amounts of EF-1 were incubated for 10 min at 27 °C in the presence and absence of tRNAs and GTP (25 μM) in a 100-μl reaction mixture containing 25 mM Tris-HCl, pH 7.5, 100 mM NaCl, 1 mM $MgCl_2$, 5 mM 2-mercaptoethanol, and 10% (v/v) glycerol. The mixture was then separated at 27 °C on a calibrated Superose-6 FPLC column (1 × 30 cm; Pharmacia, Uppsala), equilibrated with the same buffer, and containing 25 μM GTP when indicated. Fractions were analyzed for EF-1 activity and by SDS-PAGE. The amount of the different subunits of EF-1 in each fraction was estimated from Coomassie-stained gels via microdensitometric scanning. Phenylalanine-specific tRNA from yeast (Boehringer) was aminoacylated as described (33). To estimate the void volume (V_0) of the Superose 6 column, ferritin (1 mg/ml) was cross-linked for 3 h at 37 °C in the presence of 2.5 mM glutaraldehyde (38). Subsequent analysis on Superose 6 showed the gradual formation of a stable high molecular weight form of ferritin, eluting with a sharp leading edge at 7.8 ml.

TABLE I
Purification of EF-1 from Artemia

Purification step	Total protein[a]	Specific activity[b]	Yield[c]
	mg	*units/μg*	%
Crude extract (S-30)	15,000	1.7	100
Two phase separation[d]	1,100	7.0	31
Hydroxyapatite[e]	22	36	3.2
Sucrose gradient			
11.6 S[f]	5.5	80	1.8
7.6 S	4.8	65	1.3

[a] Starting from 425 g of *Artemia* cysts.
[b] One unit of activity is defined as the amount of protein required to bind 1 pmol of Phe-tRNA to ribosomes. With 2.5% of the crude extract protein being EF-1, it can be estimated that as expected the specific activity of EF-1 in the extract is similar to that of purified EF-1.
[c] Yield on basis of EF-1α activity. 70% of the total cellular EF-1α should be in a form different from the complex with EF-1βγδ and EF-1βγ, because in *Artemia* extracts there is a certain excess of EF-1α above the maximal stoichiometric amount present in EF-$1\alpha_2\beta\gamma\delta$ (see Table II, also for the estimate of 70%). Of the 30% of EF-1α present in complex, at least half and probably more would dissociate from EF-1βγδ and EF-1βγ during isolation (K_{diss} in the order of 10^{-5} M^{-1}; from Ref. 25 and Fig. 6). Therefore the 3% yield of high molecular weight EF-1 is in the range expected for this population distribution of EF-1α. Indeed, upon hydroxyapatite chromatography most of the EF-1α has been found well separated from the complexed form (see "Discussion" for the nature of this non-EF-1 bound EF-1α).
[d] This step includes ammonium sulfate fractionation and dialysis.
[e] This step includes concentration on hydroxyapatite and vacuum dialysis.
[f] S values refer to sedimentation data as obtained in Fig. 1.

RESULTS

Isolation and Composition of EF-1—The original procedure to prepare EF-1 (23) has been improved leading to the development of a fast and reliable method for obtaining milligram amounts of pure EF-1 in a single round of purification (a typical result is shown in Table I). Special attention has been paid to remove all traces of artemin, a major developmentally-regulated protein present in *Artemia* cysts (39). The purification procedure has been repeated more than 10 times, each time with similar results. From Fig. 1 it is evident that starting from *Artemia* cysts, two distinct complexes of EF-1, complex I (peak fraction number 7) and complex II (peak fraction number 10), can be isolated, free from the faster sedimenting peak of artemin (peak fraction number 2). The two complexes of EF-1 both contain the EF-1α, EF-1β, and EF-1γ subunits, but differ by the presence of an additional 28-kDa protein in one of them (complex I). Originally, this 28-kDa protein was not considered to be an intrinsic part of EF-1. However, as soon as it was realized that on the basis of one-dimensional peptide mapping according to Cleveland *et al.* (40), preparations of EF-1 and EF-1βγ both contained comparable amounts of the same 28-kDa protein, and that this 28-kDa protein did possess guanine nucleotide exchange activity (20), we concluded that we were dealing here with a second type of EF-1β like in wheat germ, where also two EF-1β-type proteins were described (41, 42). Contrary to their suggestion of one EF-1β form being a degradation product of the other (42), in *Artemia*, EF-1β is clearly not a degradation product of the 28-kDa protein. The carboxyl-terminal parts of

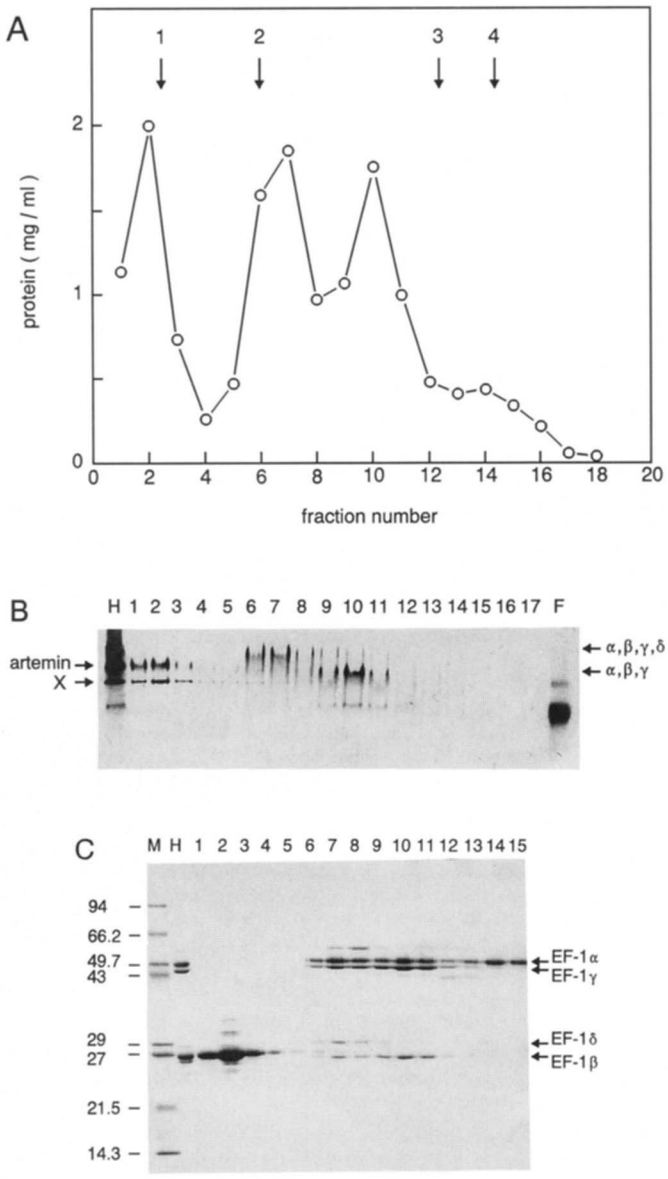

FIG. 1. **Final purification of EF-1 by sucrose gradient centrifugation.** *A*, protein profile. 100 µl of concentrated hydroxyapatite preparation (4.5 mg of protein) was applied onto a 4-ml 10–25% linear sucrose gradient, centrifuged for 17 h at 43,000 rpm, and analyzed as described under "Materials and Methods." Marker proteins, indicated by *arrows*, were run on separate gradients and their absorbance measured. *1*, urease from Jack bean (18.6 S, molecular mass 483 kDa); *2*, bovine catalase (11.7 S, molecular mass 247 kDa); *3*, bovine serum albumin (4.3 S, molecular mass 66 kDa); *4*, cytochrome *c* (1.7 S, molecular mass 13.4 kDa). *B*, PAGE under nondenaturing conditions. 1–17, aliquots of sucrose gradient fractions; *H*, hydroxyapatite preparation; *F*, ferritin marker. The composition of the different protein bands, as indicated by $\alpha\beta\gamma\delta$, $\alpha\beta\gamma$, artemin, and *X*, was analyzed by SDS-PAGE. The protein band designated as "*X*" is composed of a series of closely spaced bands of M_r 20,000–35,000 on SDS-PAGE and has been difficult to separate from EF-1 (16). Sucrose gradient centrifugation, however, resolves both forms of EF-1 without significant contamination of artemin or protein complex *X*. Free EF-1α, as present in fractions 13–15 (see *C*), does not migrate into the gel, because of its high isoelectric point (pI 8.5). *C*, SDS-PAGE pattern of fractions 1–15; *H*, hydroxyapatite preparation; *M*, marker proteins (from the top phosphorylase *a*, bovine serum albumin, EF-1α from *Artemia*, ovalbumin, carbonic anhydrase, chymotrypsinogen, trypsin inhibitor from soybean and lysozyme) with indicated $M_r \times 10^{-3}$ values on the *left*. The position of EF-1 subunits is indicated on the *right*. The 55,000 Da protein as present in fraction 8 is not considered part of EF-1.

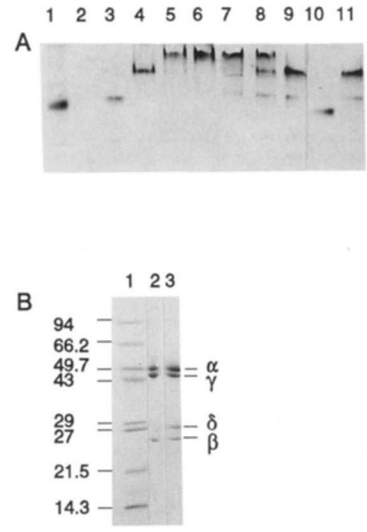

FIG. 2. **Reconstitution of two distinct EF-1 complexes is dependent on the presence of EF-1δ.** *A*, reconstitution was followed via electrophoresis under nondenaturing conditions. *Lane 1*, ferritin; *lane 2*, EF-1α (10 µg) alone. Free EF-1α does not migrate into the gel under native conditions and, hence, is not visible; *lane 3*, EF-1$\beta\gamma$ (5 µg) alone; *lane 4*, EF-1α + EF-1$\beta\gamma$; *lane 5*, EF-1α + EF-1$\beta\gamma$ + 0.5 µg of EF-1δ; *lane 6*, EF-1α + EF-1$\beta\gamma$ + 1 µg of EF-1δ; *lanes 7* and *9*, 5 µg of native isolated EF-1$\alpha\beta\gamma\delta$ and EF-1$\alpha\beta\gamma$, respectively; *lane 8*, mixture of the two isolated EF-1 complexes, 2.5 µg each; *lane 10*, EF-1δ (1 µg) alone; *lane 11*, EF-1α + EF-1δ. *B*, the composition of the major band present in *lanes 4* and *6* in *A* was analyzed by SDS-PAGE (see "Materials and Methods") and shown in *lanes 2* and *3* in *B*, respectively. *Lane 1*, marker proteins were the same as in Fig. 1*C*.

Artemia EF-1β and the *Artemia* 28-kDa protein turned out to be highly homologous, whereas the amino-terminal parts were quite different (20, 43). Therefore we designated this 28-kDa protein, a second guanine nucleotide exchange protein, as *Artemia* EF-1δ.

Reconstitution of EF-1 and the Influence of EF-1δ—The reproducible, distinct sedimentation properties of the two forms of purified EF-1, one containing EF-1δ and the other not (11.6 S and 7.6 S, respectively), argue for EF-1δ being an integral part of EF-1 rather than a contaminant. Definite evidence for a crucial role of EF-1δ in EF-1 complex formation was obtained from reconstitution experiments, monitored by electrophoresis under nondenaturing conditions. When adding an excess of EF-1α to EF-1$\beta\gamma$, one major band (Fig. 2*A*, *lane 4*) composed of α, β, and γ subunits (Fig. 2*B*, *lane 2*) was observed, whereas reconstitution in the presence of EF-1δ, under otherwise identical conditions, led to the formation of an EF-1$\alpha\beta\gamma\delta$ complex (Fig. 2*B*, *lane 3*), which migrates more slowly on nondenaturing gels (Fig. 2*A*, *lanes 5* and *6*). Furthermore, each of the two reconstituted complexes show the same electrophoretic mobility when compared to the corresponding two native forms (Fig. 2*A*, *lanes 7* and *9*). Thus in the presence of an excess of EF-1α, the formation of complex I is completely dependent on the presence of EF-1δ protein, and addition of this subunit converts complex II into complex I.

EF-1αβγδ Contains Two Molecules of EF-1α—The first indication of multiple EF-1αs present in EF-1$\alpha\beta\gamma\delta$ was obtained on comparison of the Coomassie Blue-stained gels of complex I and complex II both as isolated (Fig. 1*C*) and reconstituted (Fig. 2*B*). We found reproducibly that the EF-1α band in complex I showed about twice the intensity of that of the EF-1γ band, whereas the intensities of the EF-1α and EF-1γ bands from complex II were about the same. The basis for assuming the same color yield per µg for each subunit is that the content of basic residues, Lys, Arg, and His, which are thought to be responsible for Coomassie Blue binding (44), is about the same

TABLE II
EF-1$\alpha_2\beta\gamma\delta$ contains two molecules of EF-1α

EF-1 subunit composition	EF-1 subunit stoichiometry[a]			
	α	β	γ	δ
$\alpha\beta\gamma\delta$	1.9	1.0	1.3	1.1
$\alpha\beta\gamma$	1.2	1.0	1.3	0
$\beta\gamma\delta$	0	1.0	1.2	0.9
$\beta\gamma$	0	1.0	1.1	0
Crude extracts[b,c]	5.0	1.0	1.4	1.0

[a] Standard deviations ranged from 4 to 17% of the values shown (n = 5).

[b] Standard deviations ranged from 5 to 30% of the values shown (n = 3). This ratio did not vary significantly during the first 22 h of *Artemia* development.

[c] Equimolar amounts of EF-1$\alpha_2\beta\gamma\delta$ and EF-1$\alpha\beta\gamma$ are found upon sucrose gradient centrifugation in a reproducible ratio, both upon their purification (Fig. 1) and also in crude cyst extracts, although the latter structures were not well defined (8). On average there should therefore be 1½ EF-1αs complexed per EF-1β. Considering also the 5-fold excess of EF-1α to EF-1β in crude extracts, it is easily seen that about 70% (100 * (5 - 1.5)/5) of EF-1α should be in a form not bound to EF-1$\beta\gamma\delta$ or EF-1$\beta\gamma$.

for all of the α, β, γ, and δ subunits, being 17, 14, 15, and 13%, respectively (calculated from Refs. 43 and 45–47). To estimate the molar ratio of the subunits of EF-1 complexes more precisely, the different complexes of EF-1 were subjected to SDS-PAGE together with series of known amounts of pure EF-1α, EF-1β, EF-1γ, and EF-1δ. Coomassie Blue-stained protein bands were scanned densitometrically, and from these density readings, molar ratios of α, β, γ, and δ, normalized on β, were derived for the different complexes. The color yield per μg of pure subunit was found to be about 70, 50, 60, and 40 for α, β, γ, and δ, respectively, and relates close to the content of basic residues for each subunit. From Table II it is clear that complex I is composed of α, β, γ, and δ subunits in a molar ratio of 2:1:1:1, whereas complex II and both guanine nucleotide exchange complexes of EF-1 show equimolar ratios. Furthermore, comparable results have been obtained for reconstituted EF-1 complexes. Thus, the presence of EF-1δ in EF-1 gives rise to a complex containing an additional molecule of EF-1α, which seems also a logical consequence of two exchange factors, EF-1β and EF-1δ, being present in one complex of EF-1.

Furthermore, normalized on a fixed amount of EF-1α, EF-1$\alpha_2\beta\gamma\delta$ is equally active as EF-1$\alpha\beta\gamma$ in stimulating poly(phenylalanine) synthesis and Phe-tRNA binding to ribosomes (Fig. 3, *B* and *C*). This means that on a molar basis, EF-1$\alpha_2\beta\gamma\delta$ is twice as active as EF-1$\alpha\beta\gamma$. Since EF-1β and EF-1δ are indistinguishable in guanine nucleotide exchange tests (Ref. 20 and Fig. 3*A*), it almost follows that the difference in activity between EF-1$\alpha_2\beta\gamma\delta$ and EF-1$\alpha\beta\gamma$ resides in two rather than one copy of EF-1α being present in EF-1$\alpha_2\beta\gamma\delta$. We conclude that the two EF-1 complexes, complex I and complex II are monomers or multimers of EF-1$\alpha_2\beta\gamma\delta$ and EF-1$\alpha\beta\gamma$, respectively.

Hydrodynamic Properties of EF-1—Since native molecular masses cannot be derived directly from the sedimentation experiments of the type used here without an additional parameter for the shape of the molecule, the two purified protein complexes EF-1$\alpha_2\beta\gamma\delta$ and EF-1$\alpha\beta\gamma$ were subjected to gel filtration to determine their Stokes radii (48). Surprisingly, both forms of EF-1 behave as a particle with the same effective Stokes radius (r_e) of 6.9 nm, which, together with the sedimentation data, indicates an asymmetric shape (f_e/f_o), different for each of the two complexes of EF-1 (Table III). Native molecular masses were calculated using a rearranged Svedberg equation

$$M = 6\pi\eta N r_e S/(1 - \bar{\nu}\rho) \quad \text{(Eq. 1)}$$

where M is molecular mass (Da), η and ρ are viscosity and density of water at 0 °C, respectively (1 g/m·s and 0.998 g/ml, respectively), N is Avogadro's number (6.022 × 10^{23}/mol), r_e is Stokes radius (m), S is sedimentation coefficient, and $\bar{\nu}$ is partial specific volume calculated to be 0.742 ml/g for both complexes from amino acid sequence data (43, 45–47, 49), as described by Cohn and Edsall (50). From the Stokes radii and sedimentation data, the native molecular masses were estimated to be 350,000 ± 50,000 and 235,000 ± 30,000 Da for EF-1$\alpha_2\beta\gamma\delta$ and EF-1$\alpha\beta\gamma$, respectively (Table II). These values are close to the chemical molecular masses of dimers being 408,000 and 246,000 Da, respectively, and suggest that we are dealing here rather with dimers of EF-1$\alpha_2\beta\gamma\delta$ and EF-1$\alpha\beta\gamma$, respectively.

Delineation of the Sites of Interaction between the Subunits of EF-1—Considering the interactions of EF-1δ, it has an affinity for both EF-1α and EF-1$\beta\gamma$, the former on the basis of its nucleotide exchange activity (20) and the latter by its associa-

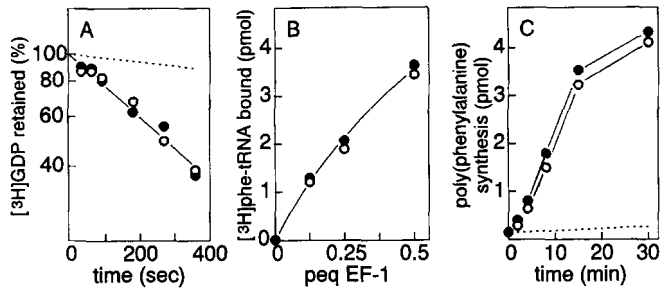

FIG. 3. **Activity of EF-1$\alpha_2\beta\gamma\delta$ (○) *versus* EF-1$\alpha\beta\gamma$ (●) in partial reaction of protein synthesis.** *A*, exchange activity was measured at 0 °C in the presence of 0.45 pmol of EF-1$\alpha_2\beta\gamma\delta$ (○) or 0.9 pmol of EF-1$\alpha\beta\gamma$ (●). A semilogarithmic plot of the time course of exchange of [^{3}H]GDP bound to EF-1α with free GDP is shown. First order rate constants were calculated to be 2.7 × 10^{-3} s^{-1} and 2.8 × 10^{-3} s^{-1} in the presence of EF-1$\alpha_2\beta\gamma\delta$ and EF-1$\alpha\beta\gamma$, respectively. The rate constant in the absence of exchange factors (– – –) was 0.3 × 10^{-3} s^{-1}. *B*, binding of [^{3}H]Phe-tRNA (20 pmol) to ribosomes (20 pmol) was measured after a 7-min incubation at 37 °C using limiting amounts of EF-1 as indicated; *peq*, pico equivalent; 1 peq of EF-1$\alpha_2\beta\gamma\delta$ = 0.5 pmol, 1 peq of EF-1$\alpha\beta\gamma$ = 1 pmol. *C*, poly(phenylalanine) synthesis was performed at 37 °C in the presence of 0.5 peq of EF-1, 2 pmol of ribosomes, and saturating amounts of [^{3}H]Phe-tRNA (30 pmol) and EF-2 (10 pmol). – – –, poly(phenylalanine) synthesis in the absence of EF-1.

TABLE III
Hydrodynamic properties of EF-1 from Artemia

Complex	s_{20}[a]	Effective radius[b] m × 10^9	Molecular mass[c]		D_{20}[d]	f/f_o[e]
			Found	Expected		
			Da × 10^{-3}			
EF-1$\alpha_2\beta\gamma\delta$	11.6 ± 0.9	6.9 ± 0.4	350 ± 50	408	3.1 ± 0.1	1.40
EF-1$\alpha\beta\gamma$	7.6 ± 0.6	6.9 ± 0.4	235 ± 30	246	3.1 ± 0.1	1.66

[a] Sedimentation coefficients for EF-1$\alpha_2\beta\gamma\delta$ or EF-1$\alpha\beta\gamma$ were determined via ultracentrifugation on sucrose gradients and comparison of their position with those of marker proteins with a known S value (see Fig. 1). The data (S *versus* fraction number) obtained for the markers were fit to a straight line by using a least-squares fitting procedure. Standard deviation was 8%.

[b] Effective Stokes radii of EF-1$\alpha_2\beta\gamma\delta$ and EF-1$\alpha\beta\gamma$ were determined via gel filtration (see "Materials and Methods"), using a linear callibration curve of Stokes radius *versus* $(K_d)^{1/3}$ obtained for the marker proteins thyroglobulin (8.3 nm), ferritin (5.99 nm), aldolase (4.6 nm), and cytochrome *c* (1.87 nm). The distribution coefficient (K_d) was calculated for each protein according to $K_d = (V_e - V_o)/(V_t - V_o)$, where V_e, V_o, and V_t are the elution positions of protein, glutaraldehyde cross-linked ferritin, and sodium chloride, respectively.

[c] Molecular mass was calculated from equations in text; the expected chemical molecular mass on the basis of a dimer.

[d] Diffusion coefficient was calculated from $D = RT/6\pi\eta N r_a$, in which R is the gas constant, and T is absolute temperature.

[e] Frictional coefficients were calculated by using $f = 6\pi\eta r$. f_o is the frictional coefficient of a hypothetical unhydrated sphere having a specified molecular mass and partial specific volume, using $r_o = (3M_c\bar{\nu}/4\pi N)^{1/3}$, where M_c is chemical molecular mass of a dimer.

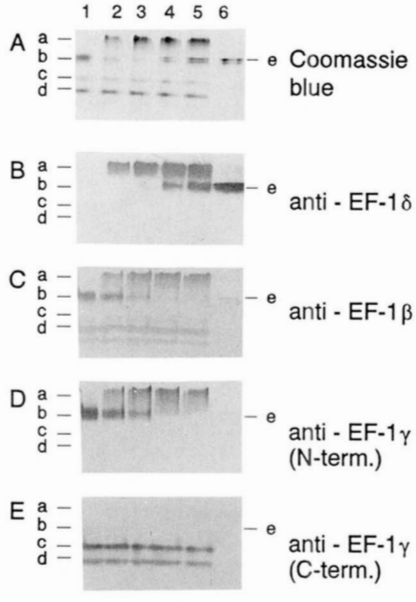

FIG. 4. **EF-1δ binds to the amino-terminal part of EF-1βγ.** Reconstitution of EF-1δ with tryptic peptides of EF-1βγ (5 μg) was monitored by electrophoresis under nondenaturing conditions. Proteins were stained with Coomassie Blue, or immunostained using monospecific antibodies toward EF-1β, EF-1γ, and EF-1δ as indicated. *Lanes 1–5*, with 0, 0.1, 0.2, 0.4, and 1.0 μg of EF-1δ, respectively; *lane 6*, 1.0 μg of EF-1δ alone. *a*, reconstituted complex of the EF-1βγ-core and EF-1δ; *b*, tryptic EF-1βγ-core composed of the amino-terminal regions of EF-1β (residues 1–103) and EF-1γ (residues 1–240), respectively; *c* and *d*, carboxyl-terminal parts of EF-1γ (residues 269–429) and EF-1β (residues 114–206), respectively. Digestion of EF-1βγ with trypsin yields a complex mixture of peptides (31), from which only two well defined carboxyl-terminal fragments *c* and *d* have been chosen. For example, the band *below band c* in *E* is also a EF-1γ-derived peptide, which migrates somewhat faster than the EF-1β-derived peptide *c*; *e*, free EF-1δ. Note that the EF-1δ subunit appears as a doublet, representing in analogy to EF-1β the nonphosphorylated and a phosphorylated form of the protein. Indeed, casein kinase II phosphorylates also EF-1δ (10, 20).

tion as a 28-kDa protein to EF-1βγ during isolation (16). Separate reconstitution experiments confirmed that EF-1δ binds both to EF-1α (Fig. 2*A*, *lanes 10* and *11*)) as well as to EF-1βγ (see Fig. 4). We have shown previously that EF-1β binds to EF-1γ by virtue of its amino-terminal part (31) and to EF-1α through its carboxyl-terminal part (20). Furthermore, the two exchange proteins EF-1β and EF-1δ show a high degree of sequence homology only in their carboxyl-terminal parts, also bearing specifically the catalytic exchange domain of EF-1β (20). Therefore, we conclude on that basis, that EF-1δ exerts its exchange function by binding to EF-1α through its carboxyl-terminal part, and as a consequence, that its amino-terminal part is bound to EF-1γ.

To determine the site of interaction of EF-1δ on EF-1βγ in more detail, EF-1δ was added to a mixture of proteolytic fragments of EF-1βγ. The mixture consisted of: 1) carboxyl-terminal fragments of both subunits, and 2) a trypsin-resistant core of EF-1βγ, which is composed of the associated amino-terminal parts of EF-1β (residues 1–103) and EF-1γ (residues 1–240), respectively (31). Reconstitution was followed by electrophoresis under nondenaturing conditions and Western blotting, using monospecific antibodies to EF-1β and EF-1δ and monoclonal antibodies recognizing the amino-terminal (mAb 39D10) and carboxyl-terminal (mAb 38C2) parts of EF-1γ, respectively. From Fig. 4 it is clear that EF-1δ binds to the amino-terminal half of EF-1βγ (*band b*) only, since addition of EF-1δ converts the amino-terminal core of EF-1βγ (*band b*) into a slower migrating complex (*band a*), whereas the mobilities of carboxyl-terminal peptides (*bands c* and *d*) remained the same. Moreover, when using limiting amounts of EF-1δ (*lanes 2* and *3*), this protein was exclusively found at the position of the reconstituted EF-1δ/EF-1βγ-core complex (Fig. 4*B*), emphasizing the efficiency of reconstitution. Although EF-1δ shows a high affinity toward the tryptic EF-1βγ core, separate reconstitution experiments failed to give a simple answer with respect to the primary binding site of EF-1δ to EF-1βγ. Reconstitution of an EF-1βδ complex failed, although each of the two separate components showed full guanine nucleotide exchange activity. In addition, we never observed a native EF-1βδ complex during isolation of EF-1. Reconstitution of EF-1γδ complex was equally unsuccessful, but in this case aggregation or precipitation of EF-1γ once separated from EF-1β (16) could have hampered efforts in this direction. The irreversible denaturation of EF-1γ when separated from EF-1β is illustrated from the failure of EF-1γ to rebind to EF-1β. However, in contrast to EF-1βγ, a complex of EF-1δγ was never seen in our *Artemia* extracts. Since EF-1δ is found in conjunction with EF-1βγ but never with EF-1β alone, the most simple scheme to explain the formation of EF-1βγδ is that EF-1β keeps EF-1γ soluble (16) and thus induces in EF-1βγ the proper folding pattern to bind EF-1δ. We infer by exclusion that EF-1γ provides the primary binding site for EF-1δ, but right now we cannot exclude that EF-1β supplies a secondary binding site for EF-1δ in the complex.

The Phosphoserine Residue Ser-89 of EF-1β Is Hidden in the Complex of EF-1αβγ—Phosphorylation of EF-1β at Ser-89 by casein kinase II inhibits its guanine nucleotide exchange activity on EF-1α (51). In order to examine the possible involvement of this serine of EF-1β in binding EF-1α, we phosphorylated EF-1βγ by casein kinase II in the presence of various amounts of EF-1α. We found that phosphorylation of EF-1β was inhibited concomitantly with the formation of an EF-1αβγ complex (31). Conversely, EF-1α protected EF-1βγ from being dephosphorylated by alkaline phosphatase (results not shown). Thus, Ser-89 of EF-1β is somehow hidden in the structure of EF-1αβγ. *Artemia* EF-1α contains in its EF-1β-binding part a sequence ^{421}GRFAVRD^{427}, which we found to be highly conserved in EF-1αs from yeast to human. Interestingly, this sequence resembles strongly region C of the Src SH_2 consensus sequence GXFL/MV/IRD/E/K (52). This sequence and in particular the arginine residue, is according to crystal structure studies critically involved in the recognition of phosphate (53). Therefore, it is tempting to speculate that, by analogy, Arg-426 of EF-1α points to the phosphate group attached to Ser-89 of EF-1β.

One and Not Two EF-1α Molecules Dissociate from EF-1 under the Influence of GTP and Aminoacyl-tRNA—To study the extent of dissociation of EF-$1\alpha_2\beta\gamma\delta$ under the influence of aminoacyl-tRNA, first varying amounts of aminoacyl-tRNA were added to EF-$1\alpha_2\beta\gamma\delta$ under conditions which favor the formation of a ternary complex of EF-1α·GTP·aa-tRNA (25). Then, the ternary complex was separated from high molecular weight EF-1 complexes by gel filtration (Fig. 5). The formation of a ternary complex is indicated by the coelution of roughly equimolar amounts of EF-1α and [^{3}H]Phe-tRNA in a fraction (fraction 33) well separated from the position of free [^{3}H]Phe-tRNA (fraction 35) and of EF-1 complexes (fraction 26–29). The results of Fig. 6 show clearly that on average a maximum of only half of the EF-1α gets released from EF-$1\alpha_2\beta\gamma\delta$ (1.05 mol of EF-1α/mol of EF-$1\alpha_2\beta\gamma\delta$; 52% of EF-1α dissociated), the effect being dependent on the presence of both GTP and aminoacyl-tRNA (see Ref. 54). Moreover, the specificity of EF-1 for aminoacylated tRNA is evident, since uncharged tRNA has little, if any effect on the dissociation of EF-1. Furthermore, under the conditions of ternary complex formation (25 μM GTP and 2 nmol of [^{3}H]Phe-tRNA), EF-1αβγ dissociates almost completely (0.85 mol of EF-1α/mol of EF-1αβγ; 85% of EF-1α dis-

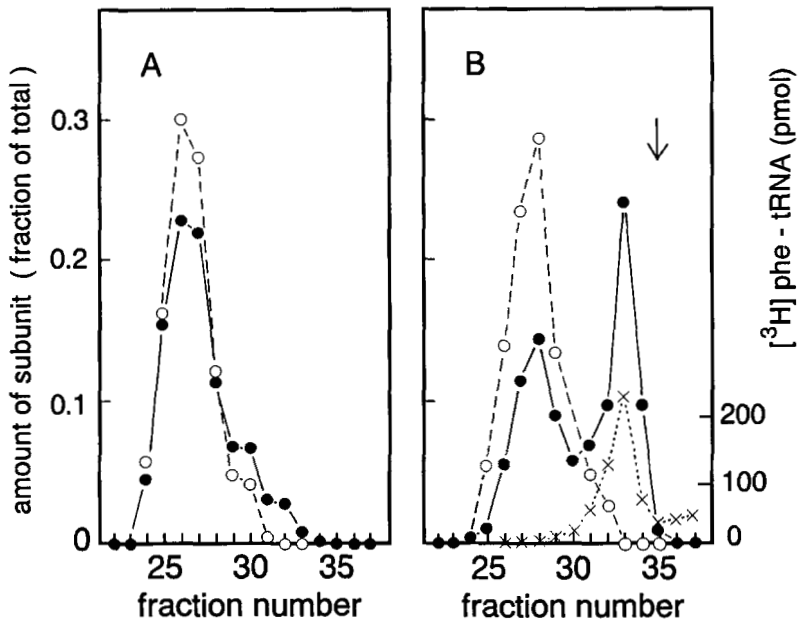

FIG. 5. **Gel filtration of EF-1$\alpha_2\beta\gamma\delta$.** Gel filtration of EF-1$\alpha_2\beta\gamma\delta$ was performed (*A*) without additions and (*B*) in the presence of both phenylalanyl-tRNA (2 nmol) and GTP as described under "Materials and Methods." Aliquots of fractions 20–40 were analyzed by SDS-PAGE. The amount of EF-1α (●) and EF-1γ (○) present in each fraction was measured via microdensitometric scanning of the Coomassie Blue-stained bands and expressed relative to the total amount of subunit present in fractions 20–40. The amount of [^{3}H]Phe-tRNA (30 cpm/pmol tRNA) (×) was determined by liquid scintillation counting. Note that there is no peak of free [^{3}H]Phe-tRNA present, most likely due to deacylation of the excess of Phe-tRNA which is not protected by EF-1α (69). Indeed, the survival of the label in [^{3}H]Phe-tRNA in the absence of EF-1$\alpha_2\beta\gamma\delta$ and GTP turned out to be low (7%) after gel filtration at 27 °C. The *arrow* indicates the position of free [^{3}H]Phe-tRNA. Note that the fractions containing EF-1$\alpha\beta\gamma\delta$ (fractions 26–29 in *B*) were also fully active in Phe-tRNA binding to ribosomes.

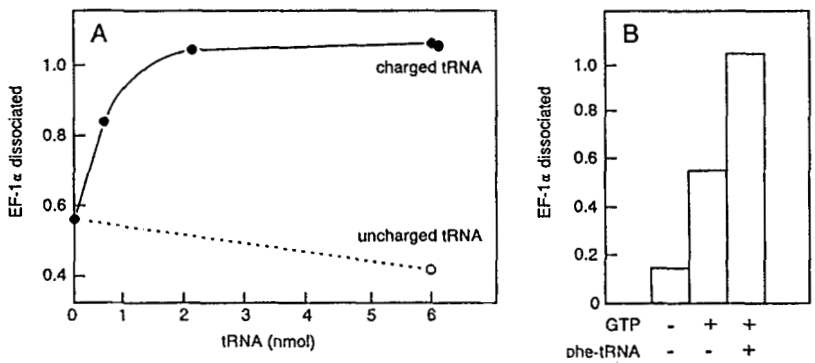

FIG. 6. **Only one molecule of EF-1α dissociates from EF-1$\alpha_2\beta\gamma\delta$ under the influence of aminoacyl-tRNA and GTP.** *A*, EF-1$\alpha_2\beta\gamma\delta$ was incubated in the presence of 25 μM GTP and varying amounts of Phe-tRNA. Dissociated EF-1α was separated from the complexed form by gel filtration as detailed under "Materials and Methods." The extent of dissociation was calculated from the amount of EF-1α present under each peak representing free EF-1α (fraction 30–35) and the complexed form (fraction 23–29), respectively (see Fig. 5). EF-1α dissociation is expressed as moles of free EF-1α found upon gel filtration per mol of EF-1$\alpha_2\beta\gamma\delta$, the latter taken as the total amount of EF-1α found in fractions 20–40 (see Fig. 5). ●, charged Phe-tRNA^{Phe}; ○, uncharged tRNA^{Phe}. *B*, in the absence of both GTP and tRNA, 8% of EF-1α was found to be dissociated from the complex. In contrast to the situation here, where the EF-1 concentration is in the order of milligrams/ml, also the second EF-1α seems to dissociate from EF-1$\alpha\beta\gamma\delta$ (as indicated from its activity in Phe-tRNA binding to ribosomes (see Fig. 5)) when the concentration of EF-1 is low (order of micrograms/ml).

sociated) into EF-1α and EF-1$\beta\gamma$, whereas EF-1$\alpha\delta$ is more stable (0.40 mol of EF-1α dissociated per mol of EF-1α; 40% of EF-1α dissociated). The observed type of Phe-tRNA-dependent EF-1α dissociation (Fig. 6) supports a sequential model with EF-1$\alpha\beta\gamma\delta$ as an intermediary product rather than a one-step model with a mixed population of only two species, EF-1$\beta\gamma\delta$ and EF-1$\alpha_2\beta\gamma\delta$. Apparently, the second molecule of EF-1α remains tighter bound to EF-1$\beta\gamma\delta$ and may follow a different path of release. The resistance of EF-1$\alpha_2\beta\gamma\delta$ to loose its second EF-1α under influence of Phe-tRNA and GTP (Fig. 6) is highly remarkable and in our opinion physiologically significant as far as the concentration of EF-1 used there is concerned. Note that EF-1 constitutes about 2.5% of the total cellular protein of *Artemia* (results not shown; see Ref. 55), corresponding to a concentration in the order of milligrams per ml in the cell.

DISCUSSION

Limited proteolysis has been used to delineate the interaction sites between the different subunits of EF-1 (Refs. 20, 31, and 32, and this paper). Together with a careful estimate of the stoichiometry of the different subunits in the EF-1 complex, it enables us to propose a model for the topography of EF-1 as depicted in Fig. 7. Our model comprises two EF-1α molecules each bound to one of the two exchange factors, EF-1β and EF-1δ, through carboxyl-terminally located regions of each of the four partners, the two copies of EF-1α, one copy of EF-1β, and one copy of EF-1δ. One "anchoring" protein, EF-1γ (16), which binds the two exchange proteins via their three amino-terminal parts, bridges the two parts of the complex, EF-1$\alpha\beta$ and EF-1$\alpha\delta$, and thereby completes the quaternary structure

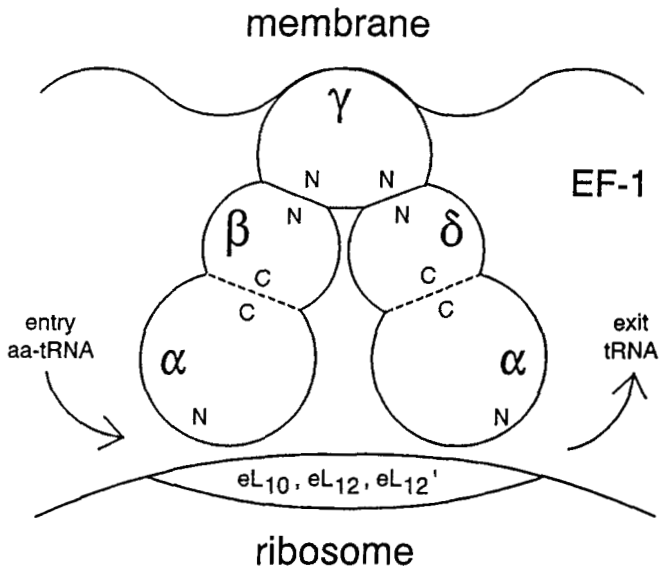

FIG. 7. **Model for the quaternary structure of elongation factor 1 and its mode of interaction on the ribosome.** The *dotted line* between the α subunit and the β, respectively, δ subunit symbolizes the transient nature of the interaction between EF-1α and the nucleotide exchange part of EF-1. In this scheme, entry and exit of tRNAs are chosen more or less arbitrarily with respect to the position of the β and δ subunit, respectively. *N* and *C* indicate amino- and carboxyl-terminally located regions of the subunits, respectively (20, 31, 32). The region of the eL10·eL12·eL12' pentameric complex on the ribosome is indicated (70).

model of EF-1. A structure like this is also found by reconstitution experiments starting from the pure subunits and is in agreement with the hydrodynamic properties of EF-1 as to size and shape (Table III; see Ref. 55). Other forms like EF-1$\alpha_2\beta_2\gamma$ and EF-1$\alpha_2\delta_2\gamma$ have never been seen, making it unlikely that we are dealing here with mixtures of the two. Definite proof for our structure of EF-1 is based on the formation of a single entity of EF-1$\beta\gamma\delta$ from EF-1$\beta\gamma$ and EF-1δ. We conclude therefore that the formation of EF-1$\beta\gamma$ creates a binding site for EF-1δ on the heterodimer and thereby also an extra one for EF-1α.

One of the main problems which is inherent also to our purification procedure for the isolation of high molecular weight EF-1, relates to the low yield of the complex in relation to the amount of EF-1α present in the cell (23, 56, 57). The preferential occurrence of high molecular weight EF-1 in *Artemia* cysts compared to nauplii, obviously makes the cyst a convenient source for the purification of this complex. Nevertheless, the fairly high dissociation constant of EF-1 even in the absence of guanine nucleotides, would bring the predicted yield only to 10–50% (see Table I). Considering also that in *Artemia* extracts the molar ratios of total α:β:γ:δ equals 5:1:1:1, or that on that score 70% of the EF-1α should be in a form not bound to EF-1$\beta\gamma\delta$ (see Table II), the obtained yield of 3% of EF-1 in the form of high molecular form EF-1 is in the range expected. We are therefore confident that the isolated forms of EF-1 represent a substantial part, that is in the order of 30% of the total EF-1α in the cell.

One important question remains, which of the two biochemical complexes of EF-1$\alpha_2\beta\gamma\delta$ or EF-1$\alpha\beta\gamma$ is the physiological one. Could it be that "stickiness" of EF-1δ to EF-1$\beta\gamma$ chains is responsible for the formation of EF-1$\beta\gamma\delta$ and that EF-1$\beta\gamma$ and EF-1δ function separately in the cell? We think the latter is unlikely. First, EF-1 complexes containing α, β, γ, and δ chains as well have been seen in a large number of different tissues and organisms (41, 42, 54, 58, 59), their precise stoichiometry although often unknown. In the case of *Artemia*, the specificity of the composition of its native and reconstituted EF-1 gives strong and additional evidence in favor of EF-1$\alpha_2\beta\gamma\delta$ being a biochemically significant form. Second, immunofluorescence studies on human fibroblasts indicate in the region of the endoplasmic reticulum the presence of substructures composed of each of the three types of proteins, EF-1β, EF-1γ, and EF-1δ. Part of EF-1α is also in the endoplasmic reticulum region but its distribution is more extended through the whole cell. The combined observations therefore strongly support the proposed structural model of EF-1$\alpha_2\beta\gamma\delta$. The presence of EF-1 at the endoplasmic reticulum is in agreement with earlier *in vitro* work, showing that EF-1γ binds specifically to membranes and tubulins (16). EF-1γ rather than EF-1α (see Ref. 60) may therefore be a candidate to anchor the complex of EF-1$\alpha_2\beta\gamma\delta$ to the endoplasmic reticulum (Fig. 7) together with ribosomes, which are known to give rise to rough membranes (61). This dual affinity of EF-1$\alpha_2\beta\gamma\delta$ to both ribosomes and membranes might thus explain a special feature of eukaryotic protein synthesis, namely that it takes place at the cell membrane. The proposed role of EF-1γ in binding EF-1 to membranes should not detract from the fact that reticulocyte EF-1 also harbors an EF-1γ type of subunit without a fully developed ER membrane system to interact with (42). Acceleration of protein synthesis by having two exchange factors together in EF-1 may be the main function of EF-1γ in reticulocytes.

In our opinion the complex of EF-1$\alpha_2\beta\gamma\delta$ should be considered as a normal reaction intermediate in the eukaryotic elongation cycle of protein synthesis. Composed of a guanine nucleotide binding (EF-1α) and a guanine nucleotide exchange (EF-1$\beta\gamma\delta$) part, the complex is dissociated through charged transfer RNA which drives the equilibrium between EF-1 and its constituent parts toward the dissociated side through the formation of a ternary complex composed of EF-1α, GTP, and aminoacyl-tRNA (62). Besides an increase in dissociation of EF-1 when going from cysts to nauplii (8, 23), we have observed recently also a concomitant change in the amount of the natural nucleic acid substrate for EF-1: *Artemia* cyst extracts, having an inherent low capacity to support protein synthesis (63) and a remarkably low level of EF-1 dissociation, turn out to have also a low level of aminoacylation of their tRNAs. In contrast, in extracts from *Artemia* nauplii known to possess a high level of endogenous protein synthesis (63) and a relative high degree of EF-1 dissociation (8, 23), the relative amounts of charged *versus* uncharged transfer RNA for the different amino acids are high.[2] These combined findings strongly suggest that the low degree of tRNA charging of cysts is responsible for the *in vivo* accumulation of the high molecular weight forms of EF-1 in the absence of protein synthesis. Presently we investigate whether mammalial cells use a similar strategy of lowering the degree of charged tRNA on entering into a quiescent state like the G_0 stage of the cell cycle. Here the importance of regulators of aminoacyl-tRNA synthetases in the activation of kinases and transcriptional activators especially comes to mind (64).

To return to EF-1, there are indications that this factor is part of a cellular superstructure, encompassing ribosomes, synthetases, tRNAs, and elongation factors (30). Our own sedimentation analysis studies have shown that in *Artemia* the ribosomes have an affinity to EF-1$\alpha_2\beta\gamma\delta$ both in the presence and the absence of charged transfer RNA and GMP-PCP. Under our conditions, all four subunits of EF-1, one more than the other, migrate together with the ribosome during sucrose gradient centrifugation (*cf.* bacterial EF-Tu·.EF-Ts, Ref. 26). Therefore in the cell, the guanine nucleotide exchange part may also be in

[2] M. Brandsma, G. M. C. Janssen, and W. Möller, manuscript in preparation.

close proximity of the ribosome so that any free EF-1α·GDP released from the ribosome reacts immediately with EF-1βγδ before EF-1α can diffuse out of the region of the ribosome into the cytosol. As a consequence, EF-1α would be bound either by aminoacyl-tRNA, the ribosome, or the exchange part of EF-1, but hardly be free. Such a behavior would explain the great instability of EF-1α in aqueous solvents, especially in the absence of EF-1βγδ, aminoacyl-tRNA, or 25% glycerol (65).

A puzzling aspect of the problem remains the cellular distribution of EF-1α. The molar ratio of total α:β:γ:δ = 5:1:1:1 as found in crude extracts of *Artemia* must mean that a substantial amount of EF-1α, at least as much as is present in the complex of EF-1α_2βγδ, is in a form different from that in the complex (Table II). Indeed, about 20% of the total cellular amount of EF-1α was found to be associated with an *Artemia* nuclear fraction. This result might indicate that EF-1α functions in nuclear transport and transcription processes and should stimulate searches for a possible role of EF-1α in the control of RNA synthesis. Finally a substantial part of EF-1α was found on actin filaments (17), a function seemingly unrelated to transfer RNA binding and protein synthesis.

The way in which EF-1α dissociates from EF-1 under influence of charged tRNA and GTP is clearly different from the situation in bacteria. Of the two EF-1αs bound to EF-1, only one is capable of forming a ternary complex with charged tRNA and GTP. This raises an intriguing question: have the two molecules of EF-1α similar or different functions in protein synthesis, perhaps one at the entry and the other at the exit site of the ribosome (66)? The alternative would be that in the evolutionary line that led from bacteria to eukaryotes, there simply was a development that led to more effective guanine nucleotide exchange by generating two exchange sites. Cross-linking studies on EF-1α may localize the respective reactive regions on the ribosome and thus contribute to the elucidation of the reaction pathways of each of the two EF-1αs. In this connection, it should be mentioned especially that there is evidence that during bacterial protein synthesis two rather than one molecule of GTP may be hydrolyzed per peptide bond (67, 68). To this end, two EF-Tus are inferred to bind per reaction cycle (67). From this point of view, the eukaryotic cell may expose a structural feature which so far is less evident in the bacterial counterpart.

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